illustrated in FIG. **7**B, using the example of an anti-rituximab antibody as a mitigating agent preventing interference from the competing drug and allowing the accurate detection of NAbs against the therapeutic protein.

[0099] Blocking antibodies against rituximab were tested for their ability to mitigate interference in the NAb assay of the present invention. Anti-rituximab antibodies were co-incubated in serum spiked with rituximab and added to a NAb assay, as shown in FIG. **7**C. Addition of anti-rituximab antibodies restored luciferase activity, eliminating the false positive NAb assay signal caused by rituximab.

[0100] These results demonstrate that the use of a mitigating agent against a competing drug can eliminate false positive NAb assay signal and allow for accurate detection of NAbs against a therapeutic protein.

Example 7. Mitigation of Cell-Based NAb Assay Interference by a Competing Drug in Clinical Samples

[0101] As shown in Example 5, many drug-naïve human samples from a clinical trial yielded false-positive NAb assay signal when tested for NAbs against a bispecific CD20×CD3 drug antibody, potentially due to the presence of a competing drug, the anti-CD20 antibody rituximab. In order to mitigate interference from rituximab, NAb assays were conducted using clinical samples with the addition of anti-rituximab blocking antibodies, as shown in FIG. **8**. Sample #1 is a control sample with low NAb assay signal. Samples #2 and #3 showed high false positive NAb assay signal. The addition of anti-rituximab antibodies eliminated the false positive NAb assay signal.

[0102] These results confirm that a residual competing drug in clinical samples, in this case rituximab, can interfere with a NAb assay and render the results of the NAb assay inaccurate. They further demonstrate that mitigating agents against a competing drug can eliminate false positive NAb assay signal in a clinical application. The use of mitigating agents against a competing drug allows for the accurate detection of NAbs against the therapeutic protein being tested.

Example 8. Ligand Binding Assay Design for Detecting NAbs Against a Therapeutic Protein

[0103] This example shows the experimental design of a ligand binding NAb assay of the invention for evaluating a therapeutic protein candidate. An exemplary embodiment of the invention comprises a target-capture ligand binding NAb assay. Briefly, samples are incubated with a biotinylated target and transferred to an avidin-coated microplate. Ruthenylated drug is added to the microplate in a subsequent step. In the absence of NAbs, ruthenium-labeled drug binds to the immobilized biotin-target, generating signal in the assay, as shown in FIG. **9**A. In the presence of NAbs, ruthenium-labeled drug cannot bind to the biotin-target, resulting in inhibition of the assay signal, as shown in FIG. **9**B.

[0104] Additional ligand binding NAb assays may be suitable for assessing NAbs against a therapeutic protein. For example, instead of a target-capture design, a ligand binding assay may be designed for drug-capture: the therapeutic protein of interest is immobilized, and the target is labeled for the generation of assay signal. FIG. **9**C illustrates a ligand binding assay design with biotinylated drug immobilized on an avidin-coated microplate, ruthenylated target generating assay signal, and NAbs against the immobilized drug blocking binding to the target and thereby inhibiting the assay signal.

Example 9. Ligand Binding NAb Assay Interference by a Competing Drug

[0105] Like the cell-based NAb assay described above, a ligand binding NAb assay may be susceptible to false positive or false negative results due to interference from matrix components. One potential source of interference is a second drug that competitively binds to the target of the therapeutic protein being tested, as shown in FIG. **10**A. For example, the drug antibodies cemiplimab, pembrolizumab and nivolumab share the same drug target, PD-1. If a clinical sample is tested for NAbs against cemiplimab, using the binding of ruthenylated cemiplimab to biotinylated PD-1 to generate signal, any residual pembrolizumab, nivolumab, or unlabeled cemiplimab in the clinical sample would competitively bind to the target, inhibiting the assay signal and causing a false positive result for the presence of NAbs.

[0106] As a proof of concept, increasing concentrations of cemiplimab, pembrolizumab or nivolumab were added to a target-capture NAb assay for NAbs against cemiplimab, as shown in FIG. **10**B. Concentrations of cemiplimab, pembrolizumab or nivolumab above 125 ng/mL inhibited signal from ruthenylated cemiplimab, producing a false positive NAb assay signal.

[0107] These results demonstrate that the presence of a competing drug can result in a false positive ligand binding NAb assay signal, and must be addressed in order to accurately detect NAbs against a therapeutic protein.

Example 10. Mitigation of Ligand Binding NAb Assay Interference by a Competing Drug

[0108] As described above, the presence of a competing drug may interfere with the binding of a therapeutic protein to its target in a ligand binding NAb assay, resulting in reduction of signal and a false positive NAb assay signal. In order to accurately detect NAbs against a therapeutic protein in the presence of a competing drug, binding of the competing drug to the mutual target must be mitigated. This is illustrated in FIG. **11**A, using the example of an anti-pembrolizumab or anti-nivolumab antibody as a mitigating agent preventing interference from the competing drug and allowing the accurate detection of NAbs against the therapeutic protein.

[0109] Blocking antibodies against pembrolizumab and nivolumab were tested for their ability to mitigate interference in the NAb assay of the invention. Anti-pembrolizumab or anti-nivolumab antibodies were co-incubated in samples spiked with pembrolizumab or nivolumab, respectively, and added to a ligand binding NAb assay, as shown in FIG. **11**B. Addition of mitigating agents against the competing drugs eliminated the false positive NAb assay signal caused by competitive binding to the target.

[0110] These results demonstrate that the use of a mitigating agent against a competing drug can eliminate false positive NAb assay signal in a ligand binding assay, and allow for accurate detection of NAbs against a therapeutic protein.

What is claimed is:

1. A method for detecting a neutralizing agent to a therapeutic protein in a sample, comprising: